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Alterations of renal function during dietary-induced hyperuricemia in the rat

HENRY W. SPENCER, WILLIAM E. YARGER and ROSCOE R. ROBINSON

Division of Nephrology, Department of Medicine, Duke University Medical Center and the Durham Veterans Administration Hospital, Durham, North Carolina

Alterations of renal function during dietary-induced hyperuricemia in the rat. Hyperuricemia was induced in rats ingesting a diet supplemented with 2½% uric acid and 5% oxonic acid (an inhibitor of hepatic uricase activity). After seven days, inulin clearance (C_{In}) and superficial nephron glomerular filtration rate (SNFR) were significantly lower than values recorded in healthy rats (C_{In}: 0.94 ± 0.10 vs. 3.61 ± 0.13 ml/min/kg of body wt; SNFR: 54.9 ± 3.2 vs. 129.7 ± 6.7 nl/min/kg of body wt). Filtration rate reduction was accompanied by an increased concentration of urate in renal tissue. Gross examination of the kidney revealed the presence of whitish streaks containing negatively birefringent crystals throughout the medulla and papilla. Histological examination revealed dilatation of the collecting ducts with flattening of the epithelium and intraluminal crystalline deposits. Intraluminal hydrostatic pressure was markedly higher than that observed in healthy rats in both proximal (21.5 ± 1.7 vs. 11.4 ± 0.3 mm Hg) and distal convoluted tubules (20.3 ± 2.0 vs. 7.6 ± 0.5 mm Hg). In another group of rats ingesting a similar diet, C_{In} was reduced to 1.49 ± 0.20 ml/min/kg of body wt. Partial or complete restoration of C_{In} toward normal was effected within seven additional days by the oral ingestion of a large volume of an alkali solution (C_{In}: 2.63 ± 0.44 ml/min/kg of body wt) or by the cessation of treatment with oxonic-uric acid (C_{In}: 4.70 ± 0.28 ml/min/kg of body wt). These results demonstrate that oxonic/uric acid-induced hyperuricemia is accompanied by severe filtration rate reduction, and they suggest strongly that intraluminal obstruction, via the deposition of uric acid, plays an important role in its pathogenesis.

Modifications de la fonction rénale au cours de l'hyperuricémie induite par l'alimentation chez le rat. Une hyperuricémie a été induite chez des rats par l'administration d'un régime auquel était ajouté 2,5% d'acide urique et 5% d'acide oxonique (un inhibiteur de l'uricase hépatique). Après 7 jours, la clearance de l'inuline (C_{In}) et le débit de filtration glomérulaire des néphrons superficiels (SNFR) sont significativement inférieurs aux valeurs obtenues chez les rats témoins (C_{In}: 0,94 ± 0,10 vs 3,61 ± 0,13 ml/min/kg poids corporel; SNFR: 54,9 ± 3,2 vs 129,7 ± 6,7 nl/min/kg poids corporel). La diminution du débit de filtration est accompagnée d'une augmentation de la concentration d'urate dans le tissu rénal. L'examen macroscopique des reins révèle la présence de bandes blanchâtres, dans la médullaire et la papille, contenant des cristaux négativement biréfringents. L'examen histologique montre une dilatation importante des canaux collecteurs avec un aplatissement de l'épithélium et des dépôts cristallins intra-luminaux. La pression hydrostatique intraluminale est considérablement plus élevée chez les rats témoins aussi bien dans le tube proximal (21,5 ± 1,7 vs 11,4 ± 0,3 mm Hg) que dans le tube distal (20,3 ± 2,0 vs 7,6 ± 0,5 mm Hg). Dans un autre groupe de rat ayant ingéré une alimentation semblable C_{In} était réduit à 1,49 ± 0,20 ml/min/kg poids corporel. Le retour partiel ou total vers l'état normal a été obtenu au terme de 7 jours d'étude supplémentaires soit au moyen de l'ingestion d'un volume important de solution alcaline (C_{In}: 2,63 ± 0,44 ml/min/kg poids corporel) soit par l'arrêt de l'administration d'acide urique et d'acide oxonique (C_{In} = 4,70 ± 0,28 ml/min/kg poids corporel). Ces résultats démontrent que l'hyperuricémie induite par les acides urique et oxonique est accompagnée d'une diminution importante du débit de filtration glomérulaire et ils suggèrent fortement que l'obstruction intra-luminale, par l'intermédiaire de la déposition d'acide urique, joue un rôle important dans sa pathogénie.

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The rapid development of severe hyperuricemia has been followed by the appearance of acute renal failure in several clinical settings, e.g., during chemotherapy for various types of leukemia. This form of acute renal failure has generally been attributed [1] to a direct or indirect function of hyperuricemia per se, including the intraluminal precipitation of uric acid within the collecting duct system and the secondary development of intrarenal hydronephrosis. Its ready reversibility in some patients via the induction of an alkaline diuresis has suggested that its pathogenesis may differ, at least in part, from that described in other forms of established acute renal failure.

Experimental studies on the pathogenesis of hyperuricemic acute renal failure have been hampered by the fact that marked hyperuricemia is difficult to achieve and sustain during the administration of exogenous uric acid in many animal species. In large part, this difficulty is due to an abundance of hepatic uricase activity in most mammalian species (with the exception of man and the higher apes); hence, in such species, an administered load of uric acid is converted rapidly to allantoin and the sustained induction of marked hyperuricemia is thereby minimized. Recently, however, Johnson, Stavric and Chartrand have reported [2] that hepatic uricase activity can be inhibited successfully in the rat by the oral administration of oxonic acid, an *s*-triazine compound. Fur-

thermore, the combined oral administration of oxonic and uric acids for several days has been shown [3, 4] to be accompanied by the subsequent appearance of sustained hyperuricemia, hyperuricosuria, an increased concentration of urate in whole kidney tissue and morphological evidence of altered renal structure (including the intraluminal deposition of crystalline material within the collecting duct system). Thus, oxonic-uric acid feeding in the rat may provide an excellent experimental setting in which the adverse renal functional response to hyperuricemia can be studied further.

The present renal clearance and micropuncture studies in the rat were initiated to define the functional response to feeding oxonic and uric acid for seven days, an arbitrarily chosen point in time when marked hyperuricemia and impaired renal function were clearly established. In addition, the reversibility of the lesion was assessed following seven additional days of dietary manipulation, including the oral ingestion of large volumes of an alkali solution, the cessation of oxonic-uric acid feeding, or both.

Methods

Experiments were carried out in 69 female Sprague-Dawley rats (total body wt: 180 to 245 g). Two experimental approaches were utilized: *1*) renal micropuncture and clearance techniques were used to examine renal function after seven full days of dietary treatment; *2*) standard renal clearance techniques alone were used to evaluate the contribution of further dietary manipulations to the reversibility of renal functional impairment following seven additional days of treatment. The various experimental protocols and the type and frequency of major experimental observations within each study group are outlined in Table 1.

Renal micropuncture and/or clearance studies in anesthetized animals after seven days of dietary treatment. Experiments of this type were conducted in 46 anesthetized animals which had been maintained on one of three dietary regimens for a full seven-day period (Table 1): *1*) group I: the daily ingestion *ad lib* of ground standard rat chow containing 5% oxonic acid and 2.5% uric acid (hereafter termed the "uric acid" diet) by 25 rats; *2*) group II: pair feeding, in which 12 additional rats were provided with ground rat chow alone in an amount equivalent to that ingested *ad lib* by a group I mate receiving the uric acid diet; *3*) group III: the daily ingestion *ad lib* of standard rat chow alone (9 rats). All animals in each of these three groups were allowed free access to tap water during the period in which they ingested the uric acid diet (days 1 through 7, inclusive); both food and water were withdrawn on the morning of the eighth experimental day (day 8) immediately prior to the performance of renal micropuncture or clearance studies or both. In many animals, including all of those in group II and their pair-fed mates in group I, total body weight was measured daily throughout the period of dietary treatment. Samples of tail vein blood for measurements of the plasma urate concentration were obtained in many animals on day 0 (immediately prior to the initiation of dietary treatment) and day 4 during the period of dietary treatment.

The clearance of inulin was measured in all rats in groups I through III (Table 1). Renal plasma flow was estimated by measuring the clearance and extraction of either ^{3}H-para-aminohippuric acid (PAH,

Table 1. Experimental protocols and type and frequency of observations[a]

Experimental groups	Total rats *N*	Diet[b]		Observations[c]					
		Days 1–7	Days 8–14	C_{In}	RPF	SNFR	Luminal pressure	Plasma urate	Tissue urate
Renal micropuncture and/or clearance studies in anesthetized rats after seven days of dietary treatment (day 8)									
I	25	Uric acid; water	—	25	17	11	14	18	11
II	12	Standard chow; pair-fed	—	12	10	5	9	10	9
III	9	Standard chow; *ad lib*	—	9	2	9	3	3	6
Clearance studies alone in awake rats *before* and *after* attempted dietary-induced reversal of established renal functional impairment (days 8 and 14)									
IV	8	Uric acid; water	Uric acid; sucrose-HCO_3 solution	8	—	—	—	8	8
V	7	Uric acid; water	Standard chow; sucrose-HCO_3 solution	7	—	—	—	7	7
VI	8	Uric acid; water	Standard chow; water	8	—	—	—	8	8

[a] C_{In} = inulin clearance; RPF = renal plasma flow calculated from the clearance and extraction of PAH or inulin; SNFR = single nephron filtration rate.

[b] Diets: *Uric acid* = standard chow containing 5% oxonic acid and 2.5% uric acid with tap water; *pair-fed* = an amount of standard chow equivalent to that ingested by a mate receiving the uric acid diet; *ad lib* = standard chow *ad libitum*; *sucrose-HCO_3 solution* = drinking water containing 5% sucrose and 0.5% $NaHCO_3$ (wt/vol).

[c] Numbers refer to number of animals in which indicated observations were made.

New England Nuclear Corp., Boston, MA; 18 rats) or ^{3}H-methoxy inulin (New England Nuclear Corp.; 11 rats). Single nephron filtration rate (SNFR) of superficial cortical nephrons and/or the intraluminal hydrostatic pressure within proximal and distal convoluted tubules were measured in 40 rats.[1]

Anesthesia was induced with Inactin (Promonta-Hamburg, Hamburg, West Germany), 100 mg/kg i.p. Each animal was placed on a heated table and a polyethylene catheter was inserted into the trachea to facilitate the maintenance of spontaneous respiration. Polyethylene catheters (PE 50) were inserted into the jugular vein and a carotid or femoral artery for sequential blood sampling and measurement of blood pressure throughout the study. The abdomen was entered via a midline abdominal incision with lateral extension beneath the left subcostal margin; indwelling polyethylene catheters (PE 50) were placed in the bladder and left ureter for timed collections of urine from the separate kidneys. When renal micropuncture was performed, the left kidney was isolated and prepared as described previously [5].

SNFR was measured in 25 animals in groups I through III. All of these animals received a priming dose of ^{3}H-methoxy inulin via an indwelling catheter in a jugular vein, followed by the constant i.v. administration (0.02 ml/min) of Ringer's lactate solution containing sufficient tritiated inulin to provide a level of radioactivity in collected samples of proximal tubule fluid which exceeded background by at least three times. Aqueous vasopressin (Pitressin, Parke, Davis and Co., Detroit, MI) was added in an amount sufficient to provide a rate of administration of 50 mU/kg of body wt/hr. The other 21 rats in groups I through III received ^{14}C-carboxyl inulin (New England Nuclear Corp.) and ^{3}H-PAH in priming doses of 1.0 and 3.0 μCi/100 g of body wt, respectively; the sustaining infusion was identical to that utilized during renal micropuncture studies with the exception that it contained sufficient ^{14}C-carboxy inulin and ^{3}H-PAH to provide an infusion rate of 2.0 and 20.0 μCi/100 g of body wt/100 min, respectively. Simultaneous renal micropuncture and clearance studies or renal clearance studies alone were initiated approximately 45 min after administration of the priming dose of PAH and/or inulin. Thereafter, three to five timed collections (20 to 45 min in duration) of urine were obtained sequentially from the separate kidneys for measurements of radioactivity and osmolality. Similar measurements were carried out on samples of arterial blood that were collected at the approximate mid-point of each urine collection. Samples of renal venous blood for measurement of ^{3}H-PAH or ^{3}H-inulin were obtained via cautious puncture (#30 G needle) of the renal vein near the hilus of the kidney at the mid-point of one to three clearance periods in 31 rats in groups I through III. At the termination of most experiments, a sample of arterial blood was collected for measurement of plasma urate concentration and the kidneys were excised, weighed and then divided in half along their long axes in the frontal plane so that the flat cut surfaces could be inspected and then studied under the microscope utilizing polarized light. Thereafter, as described below, selected portions of tissue from various regions of the parenchyma were set aside and prepared for subsequent analysis of the tissue urate concentration. In addition, tissue from some kidneys was fixed overnight in 10% neutral buffered formalin or absolute ethanol for histologic examination by routine light microscopy. Formalin-fixed tissue was stained with hematoxylin-eosin. Ethanol-fixed tissue was stained with the DeGalantha stain for urate, and unstained sections were examined with polarized light.

In the 25 rats in which tubule fluid was collected, the surface convolutions of end-proximal tubules were first identified by their proximity to vascular stars; their nature was confirmed by subsequent puncture and the intraluminal injection of one or two small oil droplets which disappeared promptly beneath the surface of the kidney and failed to reappear. Timed collection of end-proximal fluid for measurements of SNFR was carried out in two to six nephrons per kidney following the intraluminal insertion of a distal oil block approximately five to ten tubule diameters in length. When necessary, the rate of tubule fluid collection was adjusted so as to maintain the distal oil block in a constant position; however, in most instances, the tubule fluid flowed into the collecting pipet spontaneously and gentle suction was not required. Timed collections were always carried out for at least three minutes; longer collections (three to seven minutes in duration) were occasionally necessary in animals which had received the uric acid diet. The volume of collected samples was measured in a calibrated constant-bore microcapillary

[1] SNFR alone was measured in 14 of 40 rats (5 rats in group I, 2 rats in group II and 7 rats in group III); SNFR and intraluminal hydrostatic pressure were measured concomitantly in 11 of these 40 animals (6 rats in group I, 3 rats in group II and 2 rats in group III); intraluminal pressure alone was measured in 15 of 40 rats (8 rats in group I, 6 rats in group II and 1 rat in group III). Overall, SNFR was measured in 38 tubules from 11 kidneys in group I rats, 16 tubules from 5 kidneys of group II rats, and 54 tubules from 9 kidneys of group III animals. Intraluminal pressure was measured in 82 proximal and 46 distal tubules from 14 kidneys in group I rats, 74 proximal and 21 distal tubules from 9 kidneys of group II rats and 22 proximal and 3 distal tubules from 3 kidneys of group III animals.

tube and then washed into liquid scintillation vials in preparation for counting.

Intraluminal hydrostatic pressure was measured with sharpened glass micropipets filled with a solution of filtered 0.5% lissamine green dye in 0.85% saline solution which was connected by an air-filled tube to a hand-regulated water manometer. The capillarity of each pipet was measured by determining the pressure at which free aqueous fluid on the surface of the kidney first began to move into the pipet (−2 to +4 cm H_2O). Proximal and distal tubules were identified by injecting 0.05 ml boluses of a solution of filtered 10% lissamine green dye into the jugular vein and then observing the transit of the dye front along the length of surface nephrons. The micropipets were then inserted into these proximal and distal tubules. Particular care was taken to avoid injecting large quantities of the lissamine green dye solution into the tubule. The pressure in the manometer was adjusted in order to maintain the dye front near the tip of the micropipet. The intraluminal hydrostatic pressure was calculated as the difference between the reading on the manometer and the measured capillarity of each pipet.

Renal clearance studies in awake animals after seven days of the uric acid diet, and again after seven days of further dietary manipulation. These experiments were carried out in 23 rats which were first maintained on the uric acid diet for seven days in a manner identical to that outlined above for group I animals. On the morning of day 8, a renal clearance study was performed to assess the degree of dietary-induced renal functional impairment; the animals were then placed on one of three dietary programs (see following) for seven additional days (days 8 through 14, inclusive) and a second renal clearance study was carried out on the morning of day 15. The impact of the following dietary programs on the reversal of renal functional impairment was evaluated at the end of the second period of dietary treatment (day 15)—*1*) Group IV (eight rats): These animals continued to ingest the uric acid diet for seven additional days but drinking water was provided by the substitution of an aqueous solution containing 5% sucrose and 0.5% $NaHCO_3$ (wt/vol) for tap water. As before, access to the new sucrose-bicarbonate solution was permitted *ad lib. 2*) Group V (seven rats): The uric acid diet was discontinued and replaced by a standard diet of ground rat chow; drinking water was provided by free access to a sucrose-bicarbonate solution instead of tap water. *3*) Group VI (eight rats): The uric acid diet was discontinued and the animals were returned to a standard diet of ground rat chow with continued access to tap water *ad lib.*

Prior to the performance of each clearance study on days 8 and 15, the animals were lightly anesthetized with ether. Polyethylene catheters were then placed in a femoral artery and vein and an indwelling catheter was introduced into the bladder via the urethra. The animals were allowed to awaken and were then placed in a metal restraining cage to restrict their mobility. Sixty minutes after recovery from ether anesthesia, an i.v. priming dose of ^{3}H-methoxy inulin (1.0 μCi/100 g of body wt) was followed by the administration of a maintenance infusion (0.02 ml/min) containing sufficient ^{3}H-inulin in Ringer's lactate solution to provide a rate of administration of 2.0 μCi/100 g/100 min. After a 45-min period of equilibration, three sequential urine collections of 15 to 20 min duration were obtained; samples of arterial blood were again obtained at the approximate midpoint of each urine collection period. The radioactivity of these samples of plasma and urine was measured to determine inulin clearance as an index of the glomerular filtration rate (GFR) of both kidneys.

Upon completion of the first clearance study on day 8, the animals were then lightly anesthetized with ether and the indwelling catheters were removed. The femoral artery and vein were ligated and all skin wounds were repaired surgically. Each animal received chloramphenicol (Chloromycetin), 10 mg daily i.m., for the duration of the second period of dietary treatment. Clearance studies were again carried out in an identical fashion on day 15. Upon completion of the second study, the animals were killed and the kidneys were excised, weighed, inspected visually and then prepared for analyses of tissue urate concentration or light microscopy or both.

Analytical methods and procedures. Plasma and tissue urate concentrations were determined by the uricase method of Kageyma [6]. Prior to tissue analysis, portions of the cortex, inner and outer medulla and papilla were excised, air-dried for one week and then dried further to a constant weight at 60°C as described by Cannon, Symchyck and De Martini [7].

The radioactivity of tubule fluid and appropriate aliquots of urine and arterial and renal venous plasma was determined in a liquid scintillation system (Iso/Cap 300, Searle Analytical, Inc., Des Plaines, IL). Each sample, including those of tubule fluid, was introduced into a counting vial containing 5 ml of the following solution: 0.015% saturated stannous chloride solution, 4.2% Liquiflor (New England Nuclear Corp.), 10% Bio-Solv BBS-3 (Beckman Instruments, Inc., Fullerton, CA) in toluene. Samples of tubule fluid were counted for 100 min.

The osmolality of urine and plasma was deter-

mined with a vapor pressure osmometer (Wescor, Inc., Logan, Utah).

Calculations. Glomerular filtration rate was estimated by the clearance of ^{3}H- or ^{14}C-inulin. Renal plasma flow was estimated from the clearance of ^{3}H-PAH or ^{3}H-methoxy inulin divided by the appropriate renal extraction ratio. Single nephron filtration rate was determined as follows:

$$SNFR = TF/P_{In} \times (\text{flow rate of tubule fluid}),$$

where TF/P_{In} is the ratio between the concentration of inulin in tubule fluid and plasma.

All data are expressed as the mean ± SEM. The statistical significance of the difference between group means for both paired and unpaired data was analyzed by Student's *t* test and by an analysis of variance utilizing Duncan's test [8].

Results

Effect of the uric acid diet on the plasma urate concentration. The daily ingestion of the uric acid diet for seven days was accompanied by the appearance of marked hyperuricemia. The sequential response of the plasma urate concentration during this seven-day period of dietary treatment is summarized in Table 2. On the average, in the 41 rats so treated (groups I, IV, V and VI), in which measurements were obtained, the plasma urate concentration rose progressively from an initial treatment value of 1.36 mg/100 ml on day 0 to a final figure of 8.13 mg/100 ml on the morning of day 8 (range: 2.45 to 23.4 mg/100 ml). The latter value was significantly higher ($P < 0.001$) than that observed (2.03 mg/100 ml) on day 8 in a combined group of 13 rats (ten rats, group II; three rats, group III) which were maintained on a standard diet of ground chow alone.

Morphological observations on kidneys from animals ingesting the uric acid diet for seven days. Visual inspection of the flat-cut surface of kidneys from rats which had consumed the uric acid diet for seven days revealed (Fig. 1) the presence of white or yellow streaks throughout the papilla and medulla which occasionally extended into the cortex. On closer inspection, these streaks were found to be composed of somewhat friable deposits of crystalline and amorphous material which was birefringent under polarized light. Individual needle-like crystals could be removed easily from the terminal openings of the papillary collecting ducts; once removed, such material exhibited the strongly negative birefringence that is typical of monosodium urate. Light microscopic examination of formalin-fixed tissue stained with hematoxylin-eosin (Fig. 2, A and B) revealed that many medullary and papillary collecting ducts were dilated and that their epithelium was somewhat flattened. Blue-staining concretions were noted within the lumina of many collecting ducts and a mononuclear cell infiltrate was seen occasionally in the nearby interstitium. However, in general, the interstitial changes observed in these rats after seven days appeared to be less marked than those reported [4] in rats which had ingested a uric/oxonic acid diet for 21 days. Examination of alcohol-fixed tissue treated with the DeGalantha stain (Fig. 2C) revealed the presence of brownish-black silver-positive deposits within and immediately around the epithelial cells lining some papillary collecting ducts. Birefringent material was also noted within these areas using polarized light (Fig. 2D). The cortex appeared to be essentially normal, except for a subtle increase in the size of Bowman's space and the occasional presence

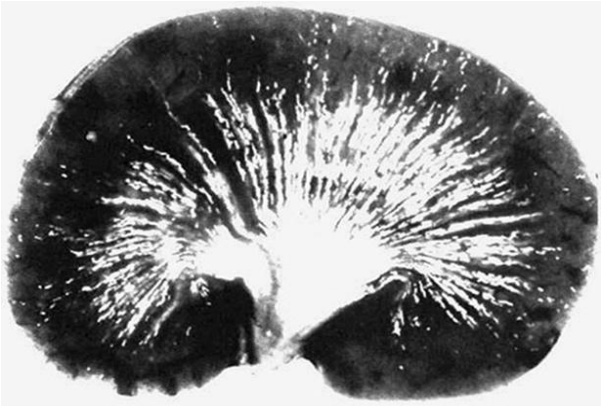

Fig. 1. *Gross appearance of the flat cut surface of a kidney from a group I rat which had ingested the uric acid diet for seven days (×10).* Note the whitish streaks throughout the medulla and papilla.

Table 2. Effect of uric acid diet on plasma urate concentration (mg/100 ml)[a]

Experimental group	Diet	Rats *N*	Experimental day 0	4	8
I, IV–VI	Uric acid	41	1.36 ± 0.11	4.93 ± 0.64	8.13 ± 0.90
II, III[b]	Standard chow	13	1.75 ± 0.31	2.39 ± 0.51	2.03 ± 0.31
P value			NS	< 0.05	< 0.001

[a] Values are mean ± SEM. NS = not significant.
[b] These 13 rats include 10 animals from group II and 3 from group III.

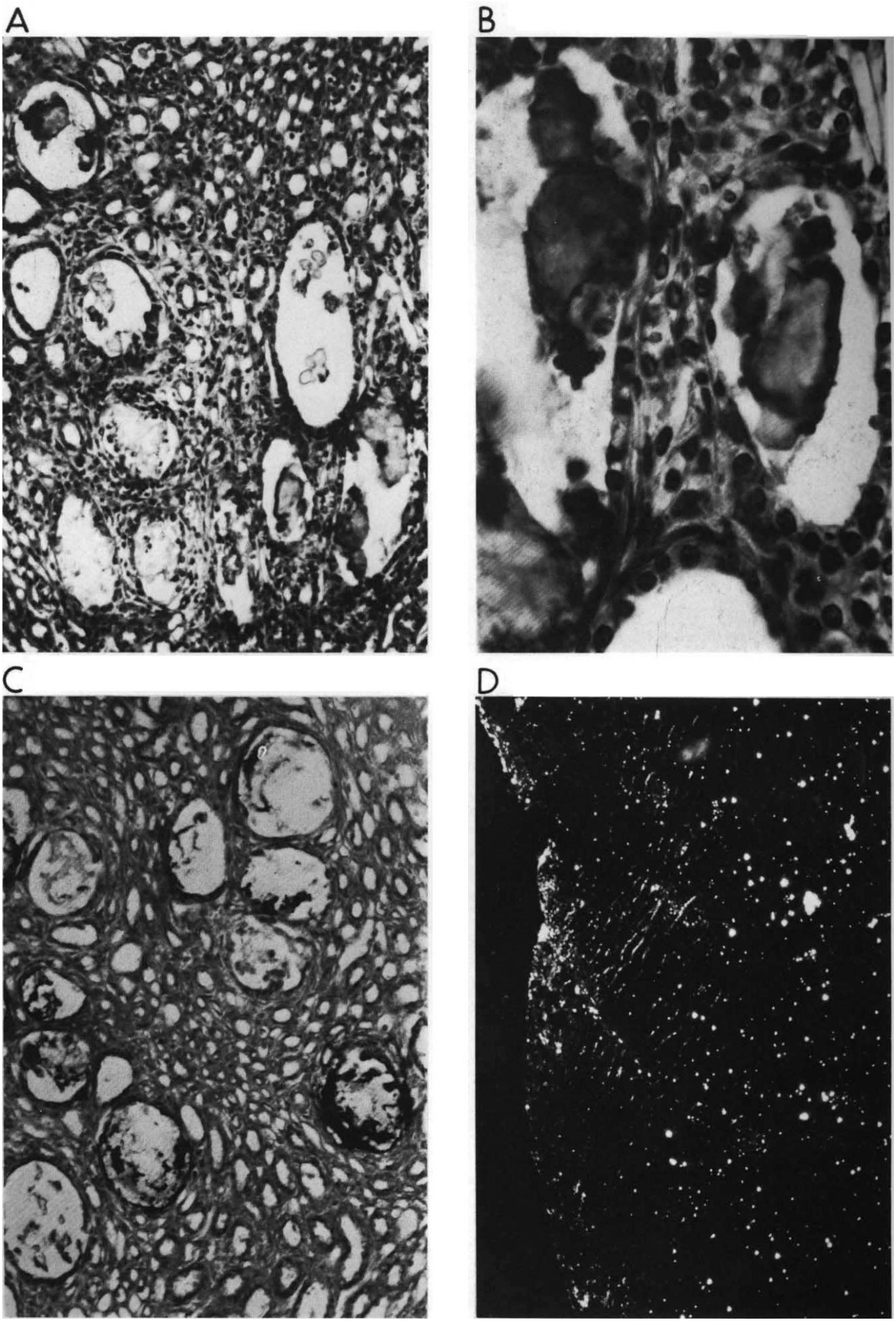

Fig. 2. *Photomicrographs of kidney tissue from a group I rat:* A (×50): Section of the papilla stained with hematoxylin-eosin. Note the luminal dilatation of collecting ducts and the presence of intraluminal concretions and interstitial infiltrate. B (×200): Higher magnification of the same area demonstrating thinning and partial attenuation of the lining epithelium of the papillary collecting ducts. C (×50): Section of the papilla stained with the DeGalantha stain demonstrating silver-positive deposits within and immediately adjacent to the epithelium of the

Table 3. Clearance data in anesthetized rats after the ingestion of the uric acid or standard chow diets for seven days[a,b]

Experimental groups	Diet	Rats *N*	C_{In} *ml/min/kg*	RPF *ml/min/kg*	FF	U/P_{osm}
I	Uric acid	25	0.94 ± 0.10 (25)	4.41 ± 0.48 (17)	0.26 ± 0.02 (17)	1.51 ± 0.05 (25)
II, III	Standard chow	21	3.61 ± 0.13 (21)	9.28 ± 0.82 (12)	0.35 ± 0.02 (12)	3.13 ± 0.38 (21)
P value			< 0.001	< 0.001	< 0.02	< 0.001

[a] Values are expressed as the mean ± SEM and they are derived from the average value for all clearance periods in each individual animal. They apply to the single left kidney alone. Figures in parentheses represent the number of rats in which observations were made.
[b] C_{In} = inulin clearance; RPF = PAH clearance corrected for extraction of PAH; FF = filtration fraction; U/P_{osm} = urine to plasma osmolality ratio.

of dilated distal convoluted tubules and cortical collecting ducts.

Clearance studies in anesthetized rats receiving the uric acid diet for seven days. The average response of inulin clearance (C_{In}), estimated renal plasma flow (RPF), filtration fraction (FF) and urine osmolality (U/Posm) to seven days of dietary treatment with the uric acid diet in 25 group I rats is shown in Table 3. These results are compared with data obtained in the 21 rats which ingested a diet of ground standard chow (groups II and III). Overall, maintenance of the uric acid diet for seven days and the parallel induction of hyperuricemia were always accompanied by a marked reduction of C_{In} and RPF on day 8. In group I rats, C_{In} and RPF of the single left kidney were reduced to 26 and 48% of the values that were observed in rats maintained on standard chow alone. Similarly, FF and urine osmolality (expressed as the osmolal U/P ratio) decreased significantly to 74 and 48% of the values observed in the groups II through III animals (Table 3).

The maintenance of animals on the uric acid diet was often accompanied by a decreased intake of food. For this reason, observations on 12 of the 25 group I rats were compared with those obtained on day 8 in the 12 pair-fed rats of group II which served as their experimental mates. An equivalent degree of weight loss had occurred by day 8 in both groups (averaging −0.028 ± 0.005 kg in group I rats and −0.032 ± 0.009 kg in group II animals). Values for C_{In} and RPF for each individual animal are depicted in Fig. 3. Inulin clearance by the single left kidney was significantly decreased ($P < 0.001$) in all group I rats (1.10 ± 0.16 vs. 3.50 ± 0.12 ml/min/kg in group II rats). Similarly, RPF was also significantly reduced ($P < 0.001$) in those rats maintained on a uric acid diet (4.17 ± 0.44 vs. 9.77 ± 0.89 ml/min/kg in group II animals).

Micropuncture studies in anesthetized rats receiving the uric acid diet for seven days. At the time of micropuncture, visual inspection of the capsular surface of the kidney revealed that most tubules appeared patent and slightly dilated. Freely movable, yellowish granular precipitates were visualized in some nephron segments which were subsequently identified as distal convoluted tubules or cortical collecting ducts. The surface of the kidney appeared otherwise normal. Micropuncture observations on SNFR, late proximal TF/P inulin ratio and/or intraluminal pressure in surface convolutions of proximal and distal tubules were obtained in 19 group I rats and 21 animals from groups II and III (Table 1). The indi-

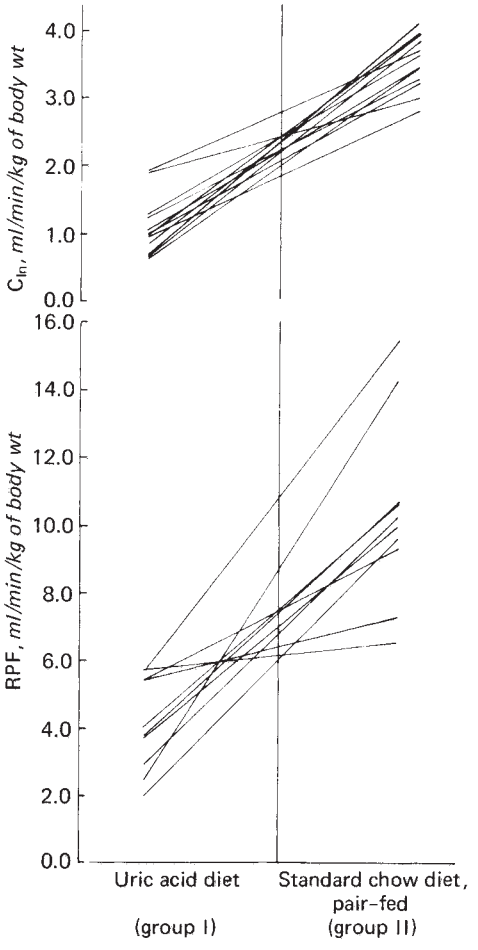

Fig. 3. *Inulin clearance (C_{In}) and renal plasma flow (RPF) by the single left kidney on day 8 in 12 group I rats receiving the uric acid diet and their 12 pair-fed mates maintained on a standard diet of ground rat chow (group II).*

Table 4. Micropuncture observations in anesthetized rats after the ingestion of the uric acid or standard chow diets for seven days[a,b]

Experimental groups	Diet	Total rats *N*	C_{In}[c] *ml/min/kg*	SNFR *nl/min/kg*	Proximal TF/P inulin	Intraluminal pressure *mm Hg*	
						Proximal	Distal
I	Uric acid	19	0.63 ± 0.10 (11)	55 ± 3 (11)	2.70 ± 0.32 (11)	21.3 ± 1.7 (14)	20.3 ± 2.0 (13)
II, III	Standard chow	21	3.76 ± 0.18 (14)	130 ± 7 (14)	2.02 ± 0.06 (14)	11.4 ± 0.3 (14)	7.6 ± 0.5 (12)
P value			< 0.001	< 0.001	< 0.005	< 0.001	< 0.001

[a] Values are expressed as mean ± SEM. Numbers in parentheses refer to number of animals in which observations were obtained.
[b] C_{In} = inulin clearance by the single left kidney; SNFR = single nephron filtration rate; TF/P inulin = tubule fluid to plasma inulin concentration ratio.
[c] These values for C_{In} were obtained only from those rats in which SNFR was measured.

vidual values from each rat were averaged and the mean expression of these average values per kidney is shown in Table 4. C_{In} by the single left kidney of the 11 group I rats (uric acid diet) in which SNFR was measured was severely reduced (−83%) when compared to that observed in animals from groups II and III (standard chow diet). This reduction of C_{In} was accompanied by an equally striking but less severe reduction of SNFR (−58%) in superficial nephrons. The disproportionately severe reduction of C_{In} and the relatively better maintenance of superficial SNFR suggests that uric acid feeding may be accompanied by a relatively greater reduction of glomerular filtration within the population of deeper nephrons. The observed relationship between superficial SNFR and C_{In} is depicted further in Fig. 4. The solid line reflects the expected relationship between these two functions if it is assumed that a single kidney contains 30,000 nephrons and that glomerular filtration is distributed evenly throughout the entire nephron population. Values for inulin clearance/superficial SNFR from individual group II and III rats (standard chow) are seen to cluster about this line whereas those from group I animals (uric acid diet) fall below, again suggesting that the SNFR of deeper nephrons may be disproportionately reduced.

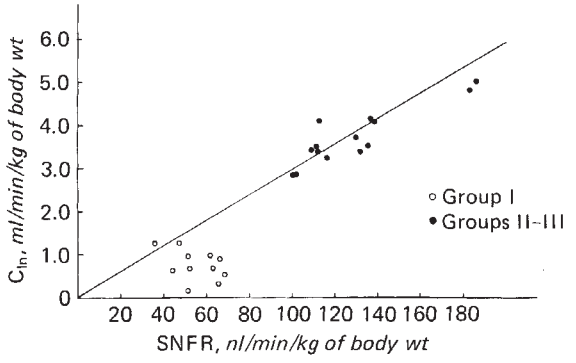

Fig. 4. *Relationship between single nephron filtration rate (SNFR) and inulin clearance (C_{In}) by the single left kidney in group I rats ingesting a uric acid diet and group II and III rats ingesting a diet of standard chow for seven days.* The *solid line* depicts the expected relationship between C_{In} and SNFR if filtration is distributed evenly throughout the entire population of nephrons.

The fractional reabsorption of glomerular filtrate was enhanced (Table 4) in late-proximal segments of superficial nephrons from rats ingesting the uric acid diet (inulin TF/P ratio: 2.70 ± 0.32 vs. 2.02 ± 0.06 in group II and III rats receiving standard chow). Importantly, the observed reduction of SNFR and elevation of fractional reabsorption in superficial proximal tubules of group I rats were also accompanied by a marked and significant ($P < 0.001$) elevation of intraluminal hydrostatic pressure in surface segments of both proximal and distal tubules (Table 4; range: 12.4 to 36.7 mm Hg in proximal tubules, 8.8 to 39.3 mm Hg in distal tubules).

The validity of these findings was strengthened further by a comparison of similar observations in pair-fed animals from groups I and II (Table 1); again, a significant ($P < 0.05$) reduction of SNFR and elevation of proximal fractional reabsorption and intraluminal pressure in proximal and distal tubules were noted in group I rats as compared to comparable observations in their pair-fed group II mates.

Intrarenal distribution of urate in animals ingesting the uric acid diet for seven days. Figure 5 portrays the average response of the renal tissue urate concentration in 11 rats in group I which had ingested the uric acid diet for seven days; it can be contrasted with that observed in 15 rats in groups II and III which were maintained on standard chow for a similar period of time. Treatment with oxonic/uric acids was accompanied by a striking increase of the tissue urate concentration within all regions of the kidney, rising steadily from an average value of 3.2 ± 0.9 mg/g of dry tissue wt in the renal cortex to a maximal figure of 23.2 ± 3.3 mg/g of dry tissue wt in the renal papilla.

Clearance studies in awake rats before and after attempted dietary-induced reversal of established renal functional impairment of seven days' duration. These studies were carried out in three groups of rats (IV

through VI; Table 1), each of which was first maintained on the uric acid diet with access to tap water *ad lib* for seven days. Thereafter, the animals were divided into three groups and clearance studies were performed on days 8 and 15 of an additional period of dietary treatment with: *1*) continued ingestion of oxonic/uric acid diet with the substitution of a sucrose-bicarbonate solution for tap water (group IV); *2*) a standard chow diet plus free access to a sucrose-bicarbonate solution for drinking water (group V); *3*) a standard chow diet and continued access to tap water alone. The influence of these three dietary regimens on an already established reduction of inulin clearance in each individual animal is depicted in Fig. 6.

On the average, for all of the 23 rats which were subsequently divided into groups IV, V or VI, the clearance of inulin was 1.49 ± 0.20 ml/min/kg per kidney on day 8 after seven days of dietary treatment with the uric acid diet and tap water. Furthermore, the average values for inulin clearance in the individual groups (IV through VI) did not differ significantly ($P > 0.05$) on day 8. Following seven additional days of dietary treatment, inulin clearance on day 15 had increased substantially and significantly ($P < 0.05$) in each group. The greatest degree of improved function was noted in groups V and VI where the restoration of a standard diet of ground chow with either sucrose-bicarbonate solution or tap water was accompanied by a marked and significant ($P < 0.05$) rise of inulin clearance to 4.55 ± 0.28 (group V) and 4.70 ± 0.28 (group VI) ml/min/kg per kidney, respectively. Both of these latter values were significantly ($P < 0.05$) higher than the average rise of inulin clearance; that was observed on day 15 in group IV rats. Nevertheless, despite the continued ingestion of the uric acid diet for seven additional days by group IV rats, the initiation of alkali treatment orally with sucrose-bicarbonate solution on day 8 was attended by a partial, but significant, restoration of C_{In} (the average value for inulin clearance rose from 1.09 ± 0.20 ml/min/kg per kidney on day 8 to 2.63 ± 0.44 ml/min/kg per kidney on day 15; $P < 0.05$).

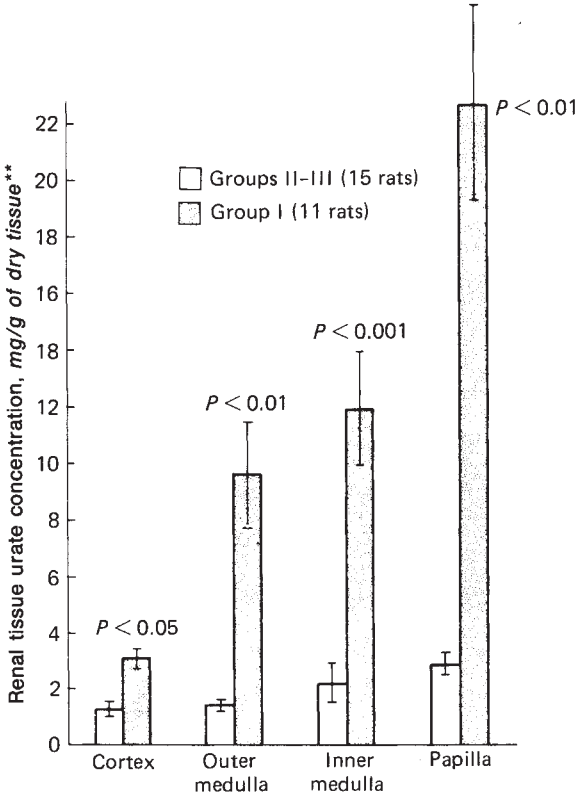

Fig. 5. *Renal tissue urate concentration in group I rats after ingesting a uric acid diet and in group II and III rats ingesting a diet of standard chow for seven days.* **Values represent mean ± SEM.

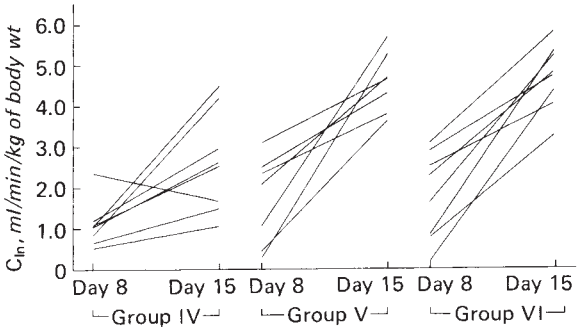

Fig. 6. *C_{In} by the single kidney before and after attempted dietary-induced reversal of established renal functional impairment.* Group IV ($N = 8$), continued ingestion of the uric acid diet plus sucrose-bicarbonate drinking water; group V ($N = 7$), restoration of standard dietary intake of ground chow plus access to sucrose-bicarbonate solution; group VI ($N = 8$), restoration of standard dietary intake of ground chow with tap water. The C_{In} of single kidneys was estimated by dividing the clearance for both kidneys by two.

Morphological appearance and the intrarenal distribution of urate on day 15. The presence of linear yellow or white streaks within the papilla or medulla was not observed in rats from groups IV through VI in contrast to the appearance of the cut surface of the kidneys from group I rats. Histological examination revealed the continued presence of dilated papillary collecting ducts. However, a paucity of other findings was noted. There was minimal evidence of interstitial edema with little or no cellular infiltrate. Urate deposits could not be demonstrated in any location using the DeGalantha stain. The intrarenal distribution of tissue urate on day 15 (Fig. 7) in rats from groups IV through VI is compared with tissue urate concentration from healthy animals ingesting standard chow (groups II and III). Even though linear deposits of amorphous material were not observed, the measured tissue urate concentration remained elevated within

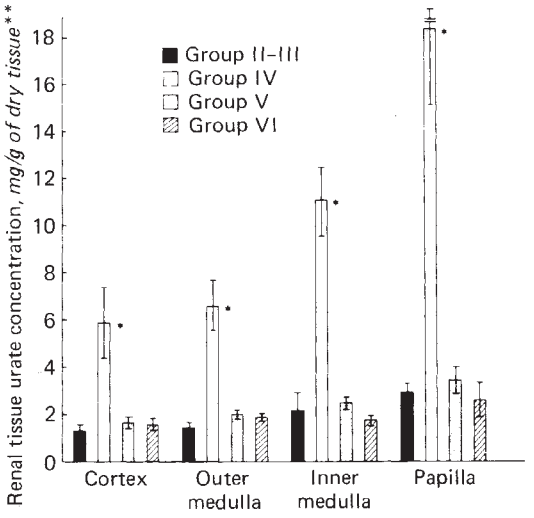

Fig. 7. *Renal tissue urate concentration following seven days of attempted dietary-induced reversal of established renal functional impairment (groups IV through VI) as compared to values from healthy rats ingesting standard chow (groups II and III).* Group IV (N = 8), continued ingestion of the uric acid diet with substitution of a sucrose-bicarbonate solution for drinking water; group V (N = 7), restoration of standard chow diet with access to a sucrose-bicarbonate solution for drinking water; group VI (N = 8), restoration of a standard chow diet with continued ingestion of tap water. **All values represent mean ± SEM. Values indicated by a single asterisk (*, group IV) differed significantly (P <0.05) from those obtained in healthy group II and III rats; none of the values for group V or VI rats differed significantly from those noted in group II and III animals.

all regions of the kidney in group IV rats which had continued to ingest the uric acid diet for seven additional days (but in which alkali treatment orally was initiated on day 8). The tissue concentrations of urate in kidneys from groups V and VI rats were significantly lower (P <0.01) than those noted in group IV rats and they did not differ significantly (P >0.05) from those observed in healthy animals, irrespective of whether or not the restoration of a standard diet was accompanied by the oral ingestion of alkali (group V) or the continued consumption of tap water (group VI).

Daily water intake and urine volume in rats ingesting a sucrose-bicarbonate solution. During the first period of dietary therapy (days 1 through 7 inclusive), water intake and the volume of urine excreted by all rats in groups IV and VI averaged 20.4 ± 0.5 and 11.6 ± 1.6 ml, respectively. In contrast, during days 8 through 14, consumption of the sucrose-bicarbonate solution averaged 124 ± 9.2 ml/day in group IV and 113.2 ± 12.7 ml/day in group V; simultaneously, the daily urine volume rose to 101.8 ± 9.6 ml/day (group IV) and 89.8 ± 18.6 ml/day (group V). Urine volume and water intake were not measured in group VI animals during days 8 through 14, but it was generally felt that these values closely resembled those observed during the initial seven days of dietary therapy.

Discussion

Previous observations in the rat have demonstrated [2–4] that the daily ingestion of a diet containing varying amounts of oxonic acid (2 to 5% wt/wt), an inhibitor of hepatic uricase activity, and uric acid (1 to 3% wt/wt) is accompanied by the subsequent appearance of hyperuricemia, hyperuricosuria, an increased urate concentration in whole kidney tissue and morphological evidence of altered renal structure. In one such study [2], 21 days of teatment with a diet containing 5.0% oxonic acid and 1.0% uric acid was followed by an elevation of the average plasma urate concentration from 0.6 to 3.5 mg/100 ml, hyperuricosuria to an average concentration of 105 mg/100 ml and elevation of the average urate concentration in kidney tissue from 5 to 72 mg/100 g of wet kidney wt. In other animals receiving the same diet [3] or one containing 2% oxonic and 3% uric acid [4], visual examination of the cut surface of the kidney revealed the presence of multiple linear whitish-yellow streaks of amorphous material which radiated through the medulla toward the tip of the papilla. Microscopic examination demonstrated the presence of birefringent crystalline material within the lumina of medullary collecting ducts and the surrounding interstitium, luminal dilatation and epithelial cell flattening of distal nephron segments and an inflammatory fibrosis throughout the medullary interstitium within which an occasional tophus and a surrounding accummulation of multinucleated giant cells was observed. Comparable hyperuricemia and renal morphological alterations were not observed when identical amounts of oxonic or uric acid were administered alone [2,4]. Importantly, observations such as these suggested that an appropriate schedule of oxonic-uric acid feeding in the rat might well afford an excellent opportunity to examine pathophysiologic mechanisms in an experimental setting which closely resembles that described in humans during rapid, severe and sustained elevation of the plasma urate concentration ("acute uric acid nephropathy").

While certain of the present observations confirm those of earlier investigators, they also offer the first description of the nature of the adverse renal functional response that may accompany oxonic uric acid-induced hyperuricemia of relatively short duration. Experimental observations were carried out after a relatively brief period of dietary treatment (seven days) with rather large amounts of oxonic and

uric acids (5 and 2.5% wt/wt, respectively) in order to examine, insofar as possible, the established renal functional response which might accompany a relatively rapid and severe rise of the plasma urate concentration over a short-term period, as opposed to that which might attend a more gradual elevation of longer duration. This objective appears to have been achieved, at least when the time course and severity of developing hyperuricemia are compared with those described by others [2–4] utilizing different dietary schedules. For example, in the present experiments, the average elevation of plasma urate concentration on days 4 and 8 of dietary treatment (4.9 and 8.1 mg/100 ml, respectively) was much more marked than that observed by Johnson et al in rats ingesting a diet containing 5.0% oxonic acid, but only 1.0% uric acid (2.2 and 3.5 mg/100 ml on days 4 and 8, respectively). The achievement of more severe hyperuricemia within a shorter period was accompanied by a renal morphological response which was almost identical to that described by others [3,4] after longer periods of dietary exposure, with the important exception that medullary interstitial fibrosis or other evidence of potentially irreversible renal damage was not yet apparent. The most striking histological alterations were confined predominantly to those that might well be regarded as potentially reversible: the intraluminal deposition of crystalline material within the collecting duct system and luminal dilatation of distal nephron segments. The exact nature of the crystalline deposits was not established in the present studies but the demonstration of negative birefringence and a positive reaction to the DeGalantha stain are features that are shared by monosodium urate. In themselves, the histological observations provide further evidence that the observed renal functional response is perhaps most analogous to that which may occur during "acute uric acid nephropathy" in humans [1], rather than that which may accompany hyperuricemia of long-standing duration.

Seven days of dietary treatment was followed by a moderately severe reduction of C_{In}, SNFR, RPF and calculated FF. C_{In} was reduced most severely, whereas the SNFR of superficial cortical nephrons and RPF, although reduced severely as well, were relatively well maintained. The greater reduction of C_{In} than superficial SNFR suggested at first glance that the reduction of filtration by deeper cortical nephrons may well have been disproportionately severe. However, the significance of an observed reduction of the GFR/SNFR ratio must be interpreted with caution in view of the fact that all measurements of SNFR were derived from proximal puncture sites; it remains possible that the insertion of a distal intraluminal oil block during the timed collection of proximal fluid may be accompanied by an artifactual elevation of SNFR within that nephron [9].

It seems unlikely that the observed renal hemodynamic alterations can be attributed directly or indirectly to contraction of the extracellular fluid volume due to a decreased intake of food and water during dietary treatment. Similar degrees of GFR and SNFR reduction were not observed in healthy pair-fed rats despite comparable degrees of weight loss over a relatively short period of time. Instead, it is of interest to note that the observed renal hemodynamic pattern (disproportionate reduction of GFR as compared to superficial SNFR, reduced FF, etc.) is not dissimilar from that described [5, 10] in various experimental forms of obstructive nephropathy in the rat, e.g., after the release of complete unilateral or bilateral ureteral obstruction of 24-hr duration. The exact cause of a disproportionate reduction of filtration by deeper cortical nephrons in any type of obstructive uropathy is unknown [5]. However, the loops of Henle of juxtamedullary nephrons descend much closer to the tip of the papilla than do the short loops of superficial nephrons (i.e., those investigated in our micropuncture studies). Inasmuch as gross deposits of amorphous crystalline material were mainly observed within the deeper regions of the medulla, it is possible that such deposits may have interfered more with the function of juxtamedullary nephrons than with the function of superficial nephrons.

The likelihood that intraluminal obstruction played an important role as a determinant of GFR and superficial SNFR reduction received strongest support from the direct observation that intraluminal hydrostatic pressure was elevated markedly within superficial cortical segments of both proximal and distal tubules. Morphological evidence of crystalline deposits within the collecting duct system and luminal dilatation of distal nephron segments offered further indirect evidence in support of such a concept. If so, these observations lend considerable credence to the long-held clinical suspicion [1] that "acute uric acid nephropathy" is due, at least in part, to the development of widespread intraluminal obstruction via the precipitation of uric acid within one or more nephron segments. According to such a thesis, any circumstance which first effects an increase in the plasma urate concentration (endogenous overproduction or, as in the present experimental setting, uric acid loading in combination with an effective inhibition of hepatic uricase activity) would set in motion a predictable sequence of events by which intraluminal obstruction would be first initiated and

then maintained: increased filtered load of urate, hyperuricosuria, precipitation of urate within distal nephron segments as a function of the interplay between the abstraction of water from tubule fluid, its acidification and concentration of uric acid, elevation of intraluminal pressure, filtration rate reduction, a further increase in plasma urate concentration and so on.

Nevertheless, despite persuasive evidence that intraluminal obstruction must contribute importantly to the observed reduction of GFR in the present experimental setting, it must also be acknowledged that still other factors may well be operative at the same or other points in time during the course of this particular form of renal injury. For example, the present observations do not exclude a contributory role of such events as epithelial cell injury and the "back leak" of tubule fluid, structural alteration of the glomerular filtration surface or an impact of vasoactive determinants of glomerular filtration dynamics, whether they be of hormonal, neurogenic or other origin. Similarly, other direct or indirect influences of hyperuricemia, an increased filtered load or tissue urate concentration cannot be excluded. These potentially adverse influences might well be independent of those that derive directly from the intraluminal precipitation of uric acid and the subsequent hydrodynamic consequences of luminal obstruction.

The present observations also demonstrate clearly that this form of acute renal injury is potentially reversible, at least when appropriate maneuvers were instituted within a single and arbitrarily selected period of time following the initiation of injury. By far the most effective reversal of filtration rate reduction was achieved by removal of the noxious stimulus itself, i.e., the cessation of dietary treatment and the subsequent resolution of hyperuricemia. Thereafter, an additional benefit of the simultaneous institution of oral alkali therapy and the initiation of a diuresis was not apparent. However, the potential therapeutic benefit attending the consumption of large volumes of an alkaline sucrose solution was underscored clearly by the observation that its institution was sufficient to effect the reversal of filtration rate reduction, at least in part, in those rats which continued to ingest the oxonic-uric acid diet. The urine pH of these rats was not measured during the period of orally administered alkali therapy; hence, the present results do not distinguish between the relative contribution of possible urinary alkalinization vs. diuresis per se to the observed improvement of filtration rate. Finally, because definite morphological evidence of intraluminal obstruction was not observed after dietary reversal therapy, it is possible that continuing elevation of the tissue urate concentration was somehow related to the incomplete reversal of filtration rate reduction. Even if valid, the nature of such a relationship is unknown.

Acknowledgments

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Reprint requests to Dr. William E. Yarger, Durham Veterans Administration Hospital, 508 Fulton Street, Durham, North Carolina 27705, U.S.A.

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